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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Dapprich
SERIAL NUMBER: 09/735,099 EXAMINER: BJ Forman
FILING DATE: December 10, 2000 ART UNIT: 1634
FOR: METHOD FOR SELECTIVELY ISOLATING A NUCLEIC ACID

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

I, Robert Ingram, of 148 North Stanworth Dr., Princeton, NJ 08540, hereby declare and state as follows:

1. I am employed by Shirley Tilghman, Department of Molecular Biology, Princeton University, a co-assignee of the above-referenced application. My title is Professional Senior Technical Staff I. I received a B.S. from Haverford College, Pa. in 1972, where I studied Biology and Chemistry.

2. I have extensive "hands-on" experience in nucleic acid sequencing, including sequencing with ABI 373 and ABI 310 automated sequencing machines, DNA cloning, gene and regulatory region knockout construction, and comparative sequence analysis. This experience is reflected in the following partial list of publications (last ten years only):

1: Bowman AB, Levorse JM, Ingram RS, Tilghman SM. Functional characterization of a testis-specific DNA binding activity at the H19/Igf2 imprinting control region. Mol Cell Biol. 2003 Nov;23(22):8345-51.

2: Sandell LL, Guan XJ, Ingram R, Tilghman SM. Gatm, a creatine synthesis enzyme, is imprinted in mouse placenta. Proc Natl Acad Sci U S A. 2003 Apr 15;100(8):4622-7. Epub 2003 Apr 01.

3: Mancini-DiNardo D, Steele SJ, Ingram RS, Tilghman SM. A differentially methylated region within the gene Kcnq1 functions as an imprinted promoter and silencer. Hum Mol Genet. 2003 Feb 1;12(3):283-94.

4: Vrana PB, Matteson PG, Schmidt JV, Ingram RS, Joyce A, Prince KL, Dewey MJ, Tilghman SM. Genomic imprinting of a placental lactogen gene in Peromyscus. Dev Genes Evol. 2001 Dec;211(11):523-32. Epub 2001 Nov 17.

5: Kurihara LJ, Semenova E, Miller W, Ingram RS, Guan XJ, Tilghman SM. Candidate genes required for embryonic development: a comparative analysis of distal mouse chromosome 14 and human chromosome 13q22. Genomics. 2002 Feb;79(2):154-61.

6: Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, Levorse JM, Tilghman SM. CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. Nature. 2000 May 25;405(6785):486-9.

7: O'Neill MJ, Ingram RS, Vrana PB, Tilghman SM. Allelic expression of IGF2 in marsupials and birds. Dev Genes Evol. 2000 Jan;210(1):18-20.

8: Shin MK, Levorse JM, Ingram RS, Tilghman SM. The temporal requirement for endothelin receptor-B signalling during neural crest development. Nature. 1999 Dec 2;402(6761):496-501.

9: Vrana PB, Guan XJ, Ingram RS, Tilghman SM. Genomic imprinting is disrupted in interspecific Peromyscus hybrids. Nat Genet. 1998 Dec;20(4):362-5. Erratum in: Nat Genet 1999 Feb;21(2):241.

10: Tilghman SM, Caspary T, Ingram RS. Competitive edge at the imprinted Prader-Willi/Angelman region? Nat Genet. 1998 Mar;18(3):206-8.

11: Webber AL, Ingram RS, Levorse JM, Tilghman SM. Location of enhancers is essential for the imprinting of H19 and Igf2 genes. Nature. 1998 Feb 12;391(6668):711-5.

12: Leighton PA, Saam JR, Ingram RS, Tilghman SM. Genomic imprinting in mice: its function and mechanism. Biol Reprod. 1996 Feb;54(2):273-8. Review.

13: Leighton PA, Saam JR, Ingram RS, Stewart CL, Tilghman SM. An enhancer deletion affects both H19 and Igf2 expression. Genes Dev. 1995 Sep 1;9(17):2079-89.

14: Leighton PA, Ingram RS, Eggenschwiler J, Efstratiadis A, Tilghman SM. Disruption of imprinting caused by deletion of the H19 gene region in mice. Nature. 1995 May 4;375(6526):34-9.

15: Tremblay KD, Saam JR, Ingram RS, Tilghman SM, Bartolomei MS. A paternal-specific methylation imprint marks the alleles of the mouse H19 gene. Nat Genet. 1995 Apr;9(4):407-13.

3. I have reviewed the November 5, 2004 Office Action. In particular, I understand that the Examiner has rejected claims 1, 3-12, 15-18, 39-40, and 42-49 as obvious over the combination of Ju et al, US Patent No. 5,876,936 ("Ju") in view of Engelhardt, US Patent No.6,221,581 ("Engelhardt"). As I understand the rejection, the Examiner contends that it would be obvious to apply the sequencing method described in Ju on genomic DNA or RNA, which is described in Engelhardt.

4. The presently claimed invention encompasses a method, which I will refer to as haplotype-specific extraction (HSE), that is performed on a population of molecules that is genomic DNA or RNA, i.e., it is performed on uncloned genomic DNA or RNA. A typical template amount used during HSE is 100 ng of DNA. Thus, for a starting mass of 100 ng human genomic DNA, about 10^4 copies of a single copy target sequence are present during the HSE reaction. In other words an entire diploid genome is fully represented about 15,000 times and every locus is represented about 30,000 times in the starting population of nucleic acids.

5. Ju describes a method of sequencing DNA that has been cloned into a plasmid or phage vector. For a typical starting mass of about 1μg plasmid DNA, about 10^{10} to 10^{11} copies of a given target sequence are expected to be present (1μg of 50kb lambda-DNA = $2x10^{10}$ molecules; 1 μg of 4kb pBR322 = $2x10^{11}$ molecules). For phage or BAC DNA typically 3-5 μg are used as starting template.

These five to six orders of magnitude difference in copy number relative to the same mass amount of DNA occur because the human genome consists of about $3x10^9$ base pairs, with the desired locus embedded in a background of millions of other sequences, compared to the much smaller size of a plasmid.

6. One of ordinary skill in the art would not have expected, at the time the instant application was filed, that Ju's sequencing method for a cloned DNA template could be successfully applied to uncloned genomic DNA or RNA. The person would have thought if a

sequencing reaction as described in Ju were to be carried out on genomic DNA, the desired locus would first need to be cloned or amplified with a method such as PCR - for the following two reasons:

A) Because of the low copy number present in the reaction, the direct use of un-amplified DNA as a template in a sequencing reaction could not be expected to provide a detectable signal for the desired locus in the sequencing equipment. As explained above, the use of genomic DNA as a direct template for a sequencing reaction would be expected to yield a signal several orders of magnitude smaller than one a plasmid-based reaction would provide, and therefore fall well below the detection limit of the sequencing system.

B) The direct use of genomic DNA as a template in a sequencing reaction does not constitute a 'clean' template in the sense that it is missing the selective purification that is achieved by cloning or during a locus-specific amplification step such as in PCR, where specificity is achieved through the interaction of two primers. In contrast, a single sequencing primer must be expected to hybridize and produce extension products for several, possibly dozens of independent loci that lead to a high, unspecific background. In the resulting detection step it would be impossible to resolve any particular sequence due to the presence of numerous other sequences at similar levels of signal. In the method described by Ju, all of the extension products generated by the use of genomic DNA as a template would also be captured and purified together - despite their differing sequences, resulting in the same problem of not being able to distinguish the desired sequence from the background of other, co-detected sequences.

There is no indication in Ju or in Engelhardt that the method described in Ju could be adapted for a target nucleic acid that has not been cloned, as is required by the claims.

The same arguments apply in kind for the use of DNA that is not cloned but has been amplified by means that are designed to provide sufficient template material of a specific locus for direct use in a sequencing reaction, such as PCR.

7. I make this declaration to rebut the Examiner's assertion, with which I do not agree. It is my opinion that one of ordinary skill in the art would not have been motivated to apply the sequencing method taught in Ju (i.e. a method designed to purify a large copy number of correctly terminated sequencing reaction products away from a background of inappropriately terminated, and thus sequence-obscuring, products) on genomic DNA as a template. Thus even combined with the knowledge of "Engelhardt", one with ordinary skill in the art, I believe, would not have devised a genomic, allele-specific enrichment and isolation method as proposed by Dapprich et al.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Robert S. Ingram

Robert S. Ingram

Signed at 2:45 PM
this 7 day of March, 2005

TRA 1836015v1